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(54) New devices and kits for immunological analysis.

(57) The invention relates to new devices and kits for immuno-assays, especially solid-phase immuno-assays, comprising a solid porous support, preferably in the form of a sheet, where antigens or immunoglobulins, or both of them, are bound by direct application, with no other chemical or electrochemical treatment. The use of such supports makes possible to effect an unlimited number of antibody-antigen reactions simultaneously and in one operation. The assays with these new kits are technically extremely simple in practice. The antigens or immunoglobulins on the solid support can be applied in any suitable pre-selected geometry, e.g. as an array of dots, preferably micro-dots, or lines. A preferred material for the solid support is nitrocellulose or nitrocellulose mixed with other cellulose esters.

Before carrying out the immuno-assays residual adsorbing sites on the support must be saturated with whole serum of heterologous species to prevent non-specific binding. The invention is also directed in particular to devices and kits treated in this manner, and, if desired, washed and dried. They can be stored for an indefinite time without loss of activity.

In the immuno-assays to be carried out according to the present-invention the preferred detection system is the use of anti-primary species antibody coupled to peroxidase with a chromogenic substrate. The color intensity can be quantitated and calibrated with standards of known amounts of immunoglobulins bound to the same support. Densitometry permits the evaluation of the color reaction over a 1000-fold range of concentrations.

The new kits can also be used with specific antibodies in a pre-determined array on the solid support for the detection of specific antigens and with complement proteins to detect antigen-antibody complexes.

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New devices and kits for immunological analysis

The present invention concerns new devices for immunological analysis, a process for their preparation, and their use, especially for multi-parameter antibody analysis and for the screening of hybridomas making monoclonal antibodies.

Tests depending directly or indirectly on the measurement of antibodies in the serum of the blood of patients are widely used in clinical diagnosis. Depending on the individual antibody in question, tests are in routine use depending on well-established principles: immune precipitate formation alone or combined with diffusion and/or electrophoresis, fixation of complement by antibody-antigen complexes, agglutination of erythrocytes by antibodies, or direct measurement of binding of the antibody to the antigen. All of these principles are used in one way or another in kits which are commercially available for the diagnosis of disease past or present. Thus, specific test kits are manufactured for the detection or measurement of antibodies which occur as a consequence of viral, bacterial, fungal or parasitic infections. Each test kit is custom-made for a specific antibody.

An inherent difficulty in the above mentioned pre-existing diagnostic kits is that false positive responses, unrelated to the disease, may result as a consequence of the high sensitivity of the procedures employed. Positive results are normally only significant when changes in the antibody titer can be detected during the progression of the disease. This prevents any rapid conclusion. A basal level for the antibody is never available for that patient prior to the particular disease, when the known test kits are used.

We understand by the term "antibody" a specific class of protein molecules characterized by being from the immunoglobulin fraction of blood or secreted by cultured cells derived from the immune system, and having a specific reaction with a corresponding ligand referred to in the text of this application by the term "antigen".

Primarily, the present invention concerns a new device for immunological analysis consisting of a porous solid support containing a pre-selected array of delimited adsorption areas of antigens, and/or of immunoglobulins, obtainable by applying aliquots of solutions or suspensions of one or more antigens or said immunoglobulins by direct contact to the support.

The absorbed areas of antigens or immunoglobulins on said solid supports can be maintained in a suitable state for reaction with antibodies or antigens, respectively, contained in a liquid, for instance a serum, which has to be analyzed, also after drying and storing the support. The invention is thus especially directed to this dried form of said device. However, before carrying out the desired immuno-assays, all the adsorption capacities for proteins on the surface of the porous support in the zones not covered by the antigens or immunoglobulins applied, and also inside these zones, must be saturated by treating the surface with non-specific proteins or sera containing such proteins. Also during this treatment the antigens or immunoglobulins originally applied are maintained intact so as to preserve the antigen-antibody reaction, and will remain so also upon drying and storing.

A second aspect of the present invention thus is a device consisting of a porous solid support containing a pre-selected array of delimited adsorption areas of antigens, and/or immunoglobulins, obtained by applying aliquots of solutions or suspensions of one or more antigens or said immunoglobulins by direct contact to the support, and further treated with excess of non-specific proteins so as to

when the device comprises antigens bound to a solid support, bound antibodies may be detected with the use of an (indicator) antibody, such as a radioactively labeled (indicator) antibody or an (indicator) antibody coupled with an enzyme giving a color reaction. By the term "indicator" a molecule which has a group attached to it which generates a detectable and measurable signal under specified conditions, is understood.

It has also been found that these immunological assays can be carried out even with extremely small dots of antigens or immunoglobulins, without interferences between the various antigens or immunoglobulins mounted and foreign substances contained in the test liquids. This finding is certainly surprising when compared to the various methods for immunological assays known in the prior art, especially those mentioned above. It is especially surprising that the simple device and the method of its application according to the present invention is of very general applicability and can be used for practically all antigenic substances including e.g. proteins, nucleic acids, carbohydrates, lipids, and related substances, and any kind of immunoglobulins.

The state of the art preceding this invention, apart from the finding in the article mentioned below in Proc. Natl. Acad. Sci. USA, which represented a decisive advance in the use of microporous sheets for the performance of antibody binding assays on replicas of electropherograms, can be exemplified by US patent No. 4,200,690 and the European patent application 27008. The US patent describes a procedure for the increasing of the binding capacity of nitrocellulose microporous supports by various complicated coating procedures in unawareness of the high intrinsic binding capacity per unit area of nitrocellulose. In addition, a number of earlier patents, summed up in the said European patent application, describe the use of various kinds of geometries of plastic surfaces with convolutions, perforations, inserts,

tended to measure the corresponding antigens. In the present invention the use of the system is possible with an essentially unlimited number of different antigens to measure their corresponding antibodies; and also the use of the support for antibodies, which will then bind their corresponding specific antigens, and the use of specific reagents which will identify antigen-antibody complexes, such as complement components.

In the article entitled "Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications", published in Proc. Natl. Acad. Sci. USA, Vol. 76, No. 9, pp. 4350-4354, September 1979, a procedure for the electrophoretic transfer of proteins from a gel to a microporous sheet and the detection of these proteins by immuno-assay procedures involving antibodies has been described. The electrophoretic transfer of the proteins give a faithful replica of the original pattern contained in the gel on a nitrocellulose sheet. The antibody assays with such transferred electropherograms are carried out after the residual adsorption capacities of the nitrocellulose sheet have been saturated by incubation with a non-specific protein, a feature which is also adopted in the present invention. The above mentioned immuno-assays with electrophoretically transferred proteins are rendered possible by the fact that no exchange takes place between the electrophoretically blotted specific proteins and the non-specific proteins used for the blocking of the residual capacity of the support. The finding of the present invention that such intact preservation of bound antigens from any interference on the part of the non-specific proteins used for the blocking of the residual adsorption sites (background adsorption) and the prolonged incubations of antibody assay is possible, also when the antigens and/or immunoglobulins are applied directly, i.e. in the absence of any electric fields, is one decisive factor for the development of the new devices and their use for antibody analysis. It is also surprising that in the further incubations with the antisera and the indicator antibody no disturbing side-reactions take place, e.g. exchange with the adsorbed

A) Natural polymeric carbohydrates and their synthetically modified, crosslinked or substituted derivatives, such as a) agar, agarose; cross-linked alginic acid; substituted and cross-linked guar gums, cross-linked dextran polymers and starches b) regenerated celluloses; cellulose esters, especially with nitric acid and carboxylic acids; mixed cellulose esters, cellulose ethers, especially with lower aliphatic alcohols.

B) Natural polymers containing nitrogen, such as proteins and derivatives, e.g. cross-linked or modified gelatin.

C) Natural hydrocarbon polymers, such as latexes and rubbers.

D) Synthetic polymers which can be prepared with suitably porous structures, such as a) vinyl polymers, such as polyethylene, polypropylene, polystyrene, polyvinylchloride, polyvinylacetate and its partially hydrolysed derivatives, polyacrylates, polyacryl-amides, polymethacrylates b) copolymers and terpolymers of the above vinyl monomers among themselves and with other monomers c) polycondensates, such as polyesters, polyamides d) addition polymers, such as polyurethanes or polyepoxides.

E) Inorganic materials which can be prepared in a suitably porous form, such as sulfates or carbonates of alkaline earth metals and magnesium, e.g. barium sulfate, calcium sulfate, calcium carbonate, magnesium carbonate, or silicates of alkali and alkaline earth metals and/or aluminium and/or magnesium, and aluminium or silicon oxides or hydrates, such as clays, alumina, talc, kaolin, zeolite, silicagel, glass. These materials can be used as such or as fillers in one of the above polymeric materials.

F) Mixtures or co-polymers of the above classes, such as graft co-polymers obtained by initiating polymerization of synthetic polymers on a pre-existing natural polymer.

miniaturized by means of procedures such as are in common practice in micro-electronics, with the use of lithographic or similar procedures, or by "charged drop" propulsion as in high-speed electronic printing, is understood. The sample can be applied so as to give any suitable geometry, the formed adsorption areas being in the form e.g. of dots, spots or lines, or any other configuration which is suitable. The array may include a great number of antigens or a few, or even a single one. There are preferably applied small volumes of the antigenic liquids or serums, for instance aliquots smaller than 1 µl, especially smaller than 0,1 µl. In this way micro-dots can be obtained on the porous surface. Microdots, for instance having a diameter smaller than 2 mm, especially smaller than 0,5 mm, are most suitable for crowding the maximum number of antigens, and/or immunoglobulins e.g. on a two-dimensional area or array; lines, e.g. of width of approximately 2 mm or less, e.g. 1 mm, may be most suitable for a more limited number of antigens or antibodies where the results can be readily visualized or quantitated by some mechanical scanning apparatus. Such an array of parallel lines can then be cut into many strips in a way which lends itself to mass production of the test system.

A typical test device according to the present invention for antibody-analysis of sera may be for example in the form as shown in the attached figure, which shows the dots as developed after immersion into the various sera to be analyzed and rendered visible by reaction with indicator antibodies coupled to enzymes capable of giving a color reaction with its substrate. Standards 1-3 are normal human serum in appropriate dilutions. Devices of these types may serve the purpose of carrying out "multi-parameter antibody" analysis. The case of kits having one single antigen mounted on the solid porous support is especially of importance for the screening of hybridomas making monoclonal antibodies.

The system of the new kits may be programmed without limits or restrictions, since any desired number of antigens or combinations of antigens or immunoglobulins can be included in a single test procedure and can

background adsorption due to remaining binding sites or exchange of the non-specific protein, it can be prevented by carrying out the incubation with the first antiserum and that with the indicator antibody in the continued presence of the same non-specific protein and additionally in the presence of total serum, as carrier, derived from species other than those of the test antibody. The continued presence of these mixtures of proteins both blocks remaining binding sites, and tends to prevent, by competition, exchange of the antibodies with proteins previously bound to non-specific sites or non-specific interaction of any kind with immunoglobulins. The carrier serum thus used should not be from a species which contains immunoglobulins which cross-react with the indicator antibody.

In the case of an immunoassay for the detection of antibodies the device prepared as described above is e.g. incubated with the antiserum to be analyzed diluted according to the expected antibody concentration, usually in the range 1:100 to 1:10000 in blocking solution, for instance in the range of 2 hours to overnight, at room temperature, and then washed extensively with physiological saline to remove excess unbound antibodies. The indicator antibody is radioactively labeled, fluorescent or luminescer[illegible] conjugated with a fluorescent substance, or with an enzyme capabl[illegible] :tion with its substrate. The indicator an[illegible] :d about 1000-fold in a mixture of the above n[illegible] incubated e.g. for two hours, and washed ag[illegible] line.

uses fluorescent techniques

These methods are carried out according to tecnniques known per se and using known indicators, including staphylococcal protein A. Thus, e.g. ^{125}I-labeled immunoglobulin can be used in autoradiography, immunoglobulin conjugated with fluorescein for the fluorometric method or with horseradish peroxidase for the enzyme immune method, with the use of o-dianisidine in the presence of hydrogen peroxide, as the substrate for the peroxidase for eliciting a color reaction, in the case of the horseradish peroxidase method, with the colored reaction product being insoluble and remaining immobilized at the site of formation.

preferably be in the form as obtained after incubation with proteins for the blocking of residual adsorption capacities, either in one or more steps. Also in this case the support, when dried, can be maintained indefinitely at the said temperature, when protected from humidity, and this form is of particular commercial importance.

In the method of using the devices of the present invention for immunological analysis, the support containing the antigens and/or immunoglobulins, after having been processed with non-specific proteins (second device), is immersed in the liquid to be analyzed, for instance serum or plasma of an animal or human patient or person in routine health care, then is dipped into a diluted solution or suspension of an indicator antibody directed against immunoglobulins of the animal species of the liquid to be analyzed, for instance anti-human immunoglobulins, such as an enzyme-coupled antibody where the enzyme reaction product is insoluble. The last step is the visualization of the bound second antibody, the preferred reaction being the oxidation of 4-chloro-1-naphthol to an insoluble color product. The last steps are sufficiently simple and rapid (the entire operation can be performed within three hours or less) that it is practicable for use in a physician's private practice.

In some cases, thorough drying of the porous support after application of the antigens is advisable or necessary. The support can be preferably air-dried for a minimum of one hour at ambient temperature. Baking is necessary for the case of nucleic acids, and it is only optional for other antigens, without being deleterious. In one particular aspect of the invention, therefore, the kits as obtained by direct application of an antigen, are baked before further processing, especially when nucleic acids antigens are included as part of the program. Baking is conveniently carried out in the temperature range from about 60° to about 120°, preferably at about 80° for a time varying from about 5 minutes to about 12 hours, e.g. for one hour. It is known from the state of the art that denatured nucleic acids bind to nitrocellulose under such conditions. It could not be anticipated that

multiple identical samples simultaneously to wells of microtiter plates. The individual wells are usually 10 cm long, 4 mm wide and 1 cm deep. The strips are incubated in this diluted solutions for example between 2 hours and overnight at room temperature with gentle agitation. The excess serum is then washed out with buffered saline, using for example 3 thorough washes. The timing of the washes is not critical. The samples of the indicator antibody are then added. This is usually a 1:1000 dilution of peroxidase coupled goat anti-human IgG (heavy plus light chain) and the treatment continued for up to 2 hours. The dilution is also usually in the same blocking solution. The indicator antibody is then washed out thoroughly by the same procedure, for example 3 times 10 minutes. The indicator antibody is then visualized by an appropriate procedure, such as fluorescence, autoradiography or suitable substrate for the coupled enzyme. In the case of peroxidase, the substrate might be O-dianisidine or chloronaphthol in the presence of hydrogen peroxide. The color reaction is then allowed to develop, for example from 30 minutes to 2 hours. The individual antibody titers are then read off by selection of the best dilution factor of the original serum, and comparison of the stain intensity with that of the standard series. The color intensity can also be read directly with some densitometric equipment, either by immersing the strip in a medium of suitable refractive index, so that it becomes transparent, and reading the transmission, or by the use of an equipment designed for measuring the reflected intensity. Equipment such as are used for measuring stain intensity on thin layer chromatograms are suitable for this latter purpose, and the same equipment can also measure fluorescence intensity. When the indicator antibody has a fluorescent label or fluorogenic substrate of an enzyme, it can also be quantitat by such equipment.

When the titer is determined with the use of a calibration series w an internal standard of e.g. a normal human serum or a pure human immunoglobulin, the unit is the fraction of total immunoglobulin wl

with which it is delivered (this protects it from cracking during cutting). The squares are placed face upward into the well of a 96 well microtiter tray (Costar Inc. Cambridge, Mass.).

(3) Blocking. To each well are added 150 µl blocking solution, which may be bovine serum albumin, whole serum or any combination. (Bovine serum albumin may be 3%, whole serum from rabbit, horse or goat may be 1-10%). It may sometimes be necessary to decomplement the serum by heating the blocking system for 30 minutes at 56°. The blocking is done for 15 minutes to 2 hours at between ambient temperature and 40°. The filters so prepared can also be stored for an indefinite time without loss of activity.

(4) Primary incubation. The blocking solution is aspirated away by the aid of a pipette, e.g. a Pasteur pipette, attached to a suction line, and the antibody test solution (primary antibody) is added. 150 µl per well is easily sufficient, but half as much will suffice. The incubation time will vary from antibody to antibody. For most purposes 2-4 hours are sufficient, but an overnight incubation may give as much as ten times more sensitivity. Antibody dilutions should be made into blocking solutions.

(5) Wash. The test antibody liquid is removed from the wells, e.g. poured out, and the support is washed at least four times, preferably with a TBS solution, and the washing duration may be anything from a few minutes to several hours.

(6) Secondary incubation. The support is incubated e.g. for 2 hours in 100 - 150 µl of horseradish peroxidase conjugated anti-"primary species" immunoglobulin with gentle shaking at room temperature, the "primary species" being that of the antibody to be tested. When, e.g., the primary antibody was raised in mouse, either peroxidase conjugated goat anti-mouse IgG, e.g. from Nordic Laboratories, Tillburg, Nether-

sheet is rewetted with TBS and a suitable number of strips is cut off at right angles to the rows of antigens, so that each strip contains one of each of the antigens. The strips are placed into 1-1.5 ml of serum dilutions in troughs of a plastic tray, manufactured by Dynatech (Alexandria, Virginia) "Disposable Reservoir Inserts". These have the same dimensions as microtiter plates and multipipetting devices (e.g. Finpipette Multichannel Pipette). The trays can be used to dilute 8 or 12 samples simultaneously. For the 8 channel insert up to 32 different antigens may be used on a 10 cm long nitrocellulose strip. For washing the liquid is first poured out from the tray and the rest is thrown out. The washing fluid is applied by filling the throughs with a wash bottle. For the secondary antibody, 1-1.5 ml is also used.

C) Quantitation. This is done with an internal standard series of either pure immunoglobulin or with whole serum containing a known amount of total immunoglobulin. The color intensity is matched against that of one of the standards either by eye, or is quantitated with the use of a scanning device. Measurement of the reflectance is done with a thin layer chromatography scanner and gives a precise quantitation with a dynamic range of three decades. The antibody concentration in the original serum is calculated from the standard curve of reflectance versus amount of immunoglobulin.

The method of anti-body analysis of the present invention shows a high degree of reproducibility of the color reaction when the indicator antibodies named above are used, and when the conditions are otherwise standardized. This color reaction is a quantitative measure of the antibody titer, when a suitable dilution of the serum or plasma is made. Furthermore, the analysis can be quantitated by adopting an internal standard series of human immunoglobulin concentrations, for instance by applying, standardized amounts of pure human IgG to the micro-porous support before the non-specific sites are blocked by the described procedure. This will give a standard color series after the test and will automatically

which will saturate the antigen. A detailed dilution series is therefore not required, which makes the invention eminently suitable for a routine use.

In some diseases, especially chronic infections, it is well known that there is a large increase in the concentration of a heterogeneous population of antibodies of a given class, but of unknown reactivity. These are called polyclonal gammopathy. Testing of serum or plasma from such a patient with a large number of randomly selected antigens will facilitate the diagnosis of such disease.

For example, the serum of a patient with infectious mononucleosis shows unexpectedly high antibody titers against 10 out of the 17 antigens used, including against the control antigens (see Example 3). Particularly high is the antibody titer against measles virus, which is antigenically unrelated, and is not previously described as having any connection with the etiology of infectious mononucleosis, which is due to infection with a totally unrelated virus. This titer is even higher than in the serum which is commercially available as a measles positive control human serum. The discovery of this unexpected high titer against measles virus will be useful in diagnosis of infectious mononucleosis. Furthermore, the detailed antibody profile will be an important new tool for the diagnosis of disease obtained with polyclonal gammopathy.

The devices described above according to the inventions are not limited to the use for antibody analysis: they can serve any objective of analytical character in biochemistry and immunology involving naturally occurring macromolecular organic substances of animal or vegetable origin, such as naturally occurring or artificially produced proteins, naturally occurring protein conjugates, such as glycoproteins, lipoproteins or protein-nucleic acid complexes, insofar as they can be applied in the said manner to porous solid supports. Immunoglobulins will also bind to the said supports, and this is why in the above

of enzymes resulting in a measurable signal.

In a recent publication, entitled "Flavin Adenine Dinucleotide as a Label in Homogeneous Colorimetric Immuno-assays" by D.L. Morris, P.B. Ellis, R. J. Carrico, F.M. Yeager, H.R. Schroeder, J.P. Albarella, R.C. Bogulaski, W.E. Hornby and R. Dawson, in the Journal "Analytical Chemistry", Volume 53, pages 658-665 (1981), a method is described where a molecule of the analyte is coupled covalently to flavin adenine dinucleotide, which is a prosthetic group for the enzyme glucose oxidase. When bound to a specific antibody, the flavin adenine nucleotide adduct with the analyte is unable to activate the glucose oxidase. When the adduct is prevented from binding to the specific antibody by the presence of the analyte, the glucose oxidase is activated, and the activation is related to the quantity of the unknown drug or hormone. The activated glucose oxidase produces H_2O_2 as a reaction product, and the H_2O_2 is a substrate for peroxidase, which can then be used together with a chromogenic substrate to yield a color reaction.

Homogeneous antibody assay systems can be readily adapted to use on a solid support according to the method of the present invention. The specific antibody and the macromolecular components of the signalling system, for example glucose oxidase and horse radish peroxidase, may be mounted by direct application on the solid porous support in any desired geometry, preferably in the same location. The coupled enzyme reactions will then benefit from their physical coincidence. According to the methods of the present invention, multiple antibodies and indicator enzymes of a signalling system can be mounted on the same support to facilitate the assay of multiple antigens simultaneously. This approach will be of great utility, for example, in drug abuse assay kits and in specific bacterial antigen identification kits.

The group of antigens which can be used with the new kits of the invention and for carrying out e.g. immunological assays is very extensive and includes e.g. human biopsy material, mammalian tissue or cells, bodily fluids, mycoplasma, metazoan parasites, fungi, bacteria, protozoa, viruses, or preparations derived from any of these. Apart from the antigens described in the illustrative Examples the following should be mentioned as being suitable to be used according to the invention: Viruses or antigens prepared form them: influenza strains, including A, A_1, A_2, B, C, parainfluenza strains 1, 2 or 3, Lymphocytic choriomeningitis virus, Mumps, Q fever Rickettsia, Rabies, Respiratory syncytial virus, Rotavirus, Rubella, Adenovirus, Eppstein Barr virus, Brucella, Hepatitis B, Cocksackie B1-B6, A9, Polio 1, 2 or 3, Reo, Echo 1-33; Fungal antigens: Histoplasmosa capsulatum, Coecidioides immitis, Blastomyces dermatitidis, Aspergillus fumigatus, flavus or carnea; Parasitic antigens: Entemeba histolytica, Trypanosoma cruzi, Echinococcus granulosis, Schistosoma mansoni; Bacterial antigens: Spirochete reiter, Treponema pallidum, Escherichia coli, Leptospira, Listeria, Salmonella, Shigella, Staphylococci, Streptococci, Legionella pneumophila; Auto-antigens: Nuclear RNP, complement fractions, Human serum proteins, Rheumatoid factor, Insulin, Insulin receptor, Thyroid stimulating hormone receptor, Acetylcholine receptor and other hormones or receptors; moreover all allergens, such as those of gramineae, e.g. Dactylis glomerata, Festuca elatior, Lolium perenne, Phleum pratense, Poa pratensis, Agristis stolonifera, Secale cereale, of herbs, e.g. Artemisia vulgaris, Chrysanthemum leucanthemum, Chenopodium album, Taraxum vulgare, Solidago virgaurea, Ambrosia trifida, of trees, e.g. Olea europea, Juglans californica, Ulmus americana, Corylus avellana, Platanus acerifolia, fungi, e.g. Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus, animals epithelia, e.g. of cats, horses, oxen, dogs or guinea pigs, of food-stuffs, e.g. milk, wheat, almonds, crabs, crevettes, of mites, of dust, of insects, e.g. of bees or wasps and of medicaments, e.g. penicillin G, penicillin V, synacthen, steroids, etc.

quantitation is to be carried out by densitometry or visually; or comprises a detection and quantitation system based on complement protein binding to antigen-antibody complexes, where the complement itself is labelled by one of the above three methods or by means of a further specific anti-complement antibody, also labelled by any one of the above three methods.

Furthermore the invention is particularly directed also to a kit and/or device, in which

a) a solid support contains an array of one or more specific antibodies and reagents for a signalling system whereby the antigen-antibody reaction results in a measurable signal, the signalling system comprising a substrate, cofactor or prosthetic group for an indicator enzyme or coupled series of enzymes, or a covalent adduct between antigen and the signalling molecule. The enzyme or coupled enzyme system can be applied together with the specific antibody or separately from it to the solid support; however, it need not be applied to the support at all, but it may be used in solution,

b) the array on the solid support contains immunoglobulins of human or animal origin, or fragments thereof, for the detection and quantitation of rheumatoid factor,

c) the array on the solid support contains complement protein for detection and quantitation of circulating antigen-antibody complexes known as circulating immune complexes.

The invention furthermore relates to the use of all the kits and devices above described, especially for the detection and quantitation of specific antigens or specific antibodies or both, by immuno-assay

treatments are performed in the well of a disposable 8 trough reservoir insert (Dynatech Laboratories, Alexandria, Virginia, USA). The incubation with the peroxidase coupled antibody is effected at room temperature during two hours with gentle agitation. The excess antibody is removed by thorough washing with TBS. Finally, the peroxidase substrate mixture is made up with 5 ml TBS, 1 µl 30% H_2O_2 in water, 10 µl o-dianisidine (1% w/v in methanol) and 1 ml of this solution is added to the trough. The reaction is then allowed to proceed for 2 hours in the dark. The excess reagents are washed out with de-ioniz
the strip air-dried at room temperature, and the size of t
measured with Vernier calipers. The following results are c

EGS.

Volume of serum	Diameter of spot
0.8 µl	1.5 mm
0.6 µl	1.3 mm
0.4 µl	1.0 mm
0.2 µl	0.6 mm
0.1 µl	0.3 mm

The diameter of the microdot is linear with respect to the volume applied when this is in the range 0-0.2 µl. It falls off from linearity at higher volumes, probably due to adsorption at the point of application. Furthermore, if Millipore sheets with a grid are used, the ink is sufficiently hydrophobic that the liquid does not spread beyond the printed squares. The intrinsic resolving power of the microdot system is clearly well below the size of the smallest volume that can be applied with common pipetting devices, namely $\leqslant$0.3 mm. A standard 100 mm length strip, fitting in the well of "trough reservoir insert", as used here, could contain 300 individual antigens spotted in a one-

Antigen used in test	Antibody titer
Sandovac vaccine (mixture)	
A/Brazil/11/78 }	625,000
A/Bankok/1/79 }	
A/Singapore/222/79 }	
Flow hemagglutination test antigens	
A/PR-8/34	2,500
A-1/FM-1/47	12,500
A-2/Hong Kong/68	< 100
A-2/England/42/72	< 100
A-2/Japan/170/62	< 100
B/Lee/40	500
B/Mass/3/66	500
Negative virus control	< 100

This shows that the serum has an extremely high titer of antibodies against the antigen with which the individual had been vaccinated, varying but significant titers against some historical influenza strains, and no detectable titer against the control antigen preparation which has no virus. Thus, all influenza strains can easily be titered against a serum of an individual in one single operation. The method is also very sensitive: endpoint at c. 10^6-fold dilution for high titer antibodies; and requires very little serum: 10 µl in 1 ml of medium for the lowest dilution. This method is also superior to the conventional hemagglutination inhibition tests or complement fixation tests, where individual assay procedures are required for each antigen. The strip also gives a permanent record of the results, and can be stored indefinitely.

Example 3: A device constructed from 10 pre-existing immunediagnostic kits

The device is constructed by spotting commercially available antigens are in Example 2. The following antigens are used with dilution factor indicated being sufficient to give the maximum response. The antigens are all diluted in TBS.

Test antigens	Test antisera	Mononucleosis	Mononucleosis control	Toxoplasma	Toxoplasma control	Measles	Measles control	Ornithosis	Herpes simplex	Mycoplasma	Adenovirus	Cytomegalovirus	Tick-borne encephalitis	Varicella zoster
Toxoplasma		-	-	(+)	-	-	-	-	-	-	-	-	-	-
Measles		+	-	+	-	(+)	-	+	-	-	-	+	-	-
Ornithosis		+	-	-	-	-	-	(+)	-	-	-	-	-	-
Ornithosis control		+	-	-	-	-	-	-	-	-	-	-	-	-
Herpes simplex		+	+	-	+	+	-	+	(+)	+	+	+	+	+
Herpes simplex control		+	-	-	-	-	-	-	-	-	-	-	-	-
Mycoplasma		+	-	-	-	-	-	+	+	(+)	+	+	-	-
Mycoplasma control		-	-	-	-	-	-	-	-	-	-	-	-	-
Adenovirus		-	+	-	+	+	-	+	+	+	(+)	+	+	+
Cytomegalovirus		+	+	-	-	+	-	+	+	+	-	(+)	+	-
Cytomegalovirus control		-	-	-	-	-	-	-	-	-	-	-	-	-
Tick-borne encephalitis		-	-	-	-	-	-	-	-	-	-	-	(+)	-
Tick-borne encephalitis control		-	-	-	-	-	-	-	-	-	-	-	-	-
Varicella zoster		+	+	-	+	+	-	+	+	+	+	+	+	(+)
Varicella zoster control		-	-	-	-	-	-	-	-	-	-	-	-	-
Influenza		+	+	+	+	+	-	+	+	+	+	+	+	+
Influenza control		+	-	-	-	+	-	-	-	-	-	+	-	-

In every case, the spot is positive for the combination of a test antigen with the corresponding test antiserum, as emphasized by the circles.

The negative control sera are all negative for the antigen for which they are provided in the purchased kits, although each may be positive for a variety of other antigens. The negative control antigens are also negative with the sera for which they are provided, although

Allergies are usually associated with the presence of circulating antibodies of the IgE type. As described here, the assay with indicator antibodies of peroxidase coupled goat anti-human IgG also detects IgM and IgE antibodies, since the goat antibody used reacts with both H and L chains. More specific tests can be constructed using antibodies which are specific for IgG or IgM, or IgE for example.

Example 4: Analysis of antibodies in the sera of patients with auto-immune and other diseases

This device is constructed with the same antigens as used in Example 3, but with the addition of antigens which are indicative of auto-immune disease. Pure and denatured nucleic acids, as described below, and subcellular fractions derived from Hela cells, which can be taken as a typical non-differentiated human cell line, and which is readily cultivated in quantity, are used. Actin and myosin are also used as antigens (rabbit: from Sigma).

Salmon sperm DNA (Serva, Heidelberg) is denatured by heating at 100°C for 2 minutes in the presence of 1M glyoxal, followed by fast cooling.

Escherichia coli ribosomal RNA is prepared from the large ribosomal subunit by well-known procedures (Gordon & Ramjoué, Analytical Biochemistry 83, 763-766 (1977)).

Hela cells are cultivated and the subcellular fractions (nuclei, mitochondria, nucleoli, polysomes and cytosol fractions) prepared from them by known procedures (Penman, S. J. Mol. Biol. 17, 117-125 (1966)).

Aliquots of 0.5 µl of all the antigens mentioned with protein concentrations in the range 1-10 mg/ml are spotted on Millipore sheets as described in Example 3, in two steps as follows:
1) fixation of the nucleic acids by direct spotting and baking of the sheet for 2 hours at 80°C, a procedure already known to cause an

	Normal		SLE					RA								SLE+RA	MCTD	MS	ICD	AE
Test	15	17	2	5	7	8	9	1	10	12	14	16	18	19	20	6	11	3	4	13
Native DNA					5															
Denatured DNA																				
RNA																				
Hela cells			5	5	5	12.5	<1	<1						1	<1					
Mitochondria			5					50												
Nuclei			5	12.5	5	12.5					<1			1	1					
Nucleoli			5		<1						<1						>500			
Cytosol			5		<1											<1	>500			
Ribosomes			5														>500			
Myosin			2		<1					1							>500			
Toxoplasma			<1	<1	<1			<1	<1	<1			<1	<1		<1	1				
Measles	<1	<1	<1	<1	<1	<1	<1	5	<1	1	<1	5	<1	1	1			<1		
Ornithosis						<1										<1	<1	<1	<1	<1
Herpes	<1	12.5	2.5			5	1	12.5	<1	10	1	10		2	5	5		12.5	<1	
Myco plasma	1	1	1											1			<1		<1	<1
Adenovirus	10	5	1	<1	1	1	1			2	1	<1	5	1	5	<1	1	5	<1	25
Cytomegalovirus	5				1	<1	1							5		2		<1		
Varicella	2	10	1	<1	2	1	1	1	12.5		1	1	5	1	1	5	12.5	1	2	10
Influenza	10	10	1	5	5	12.5	12.5	2	12.5	25	5	10	10	10	1	10	2.5	5	2	2
" control									1								1			<1

detect antibodies against these proteins. However, their substitution by the corresponding human proteins is possible, as well as by human collagen, for the diagnosis of autoimmune diseases. Furthermore, a variety of differentiated cell types making specialized proteins can also be included, as well as the undifferentiated Hela used here. This kit has the additional advantage that it can be used for the monitoring of patients sera during the course of treatment of auto-immune disease with immuno-suppressive agents. One can then monitor the disappearance of pathological anti-bodies, and regulate the treatment so as to avoid the suppression of the benign antibodies which are endemic, and thus avoid totally compromising the immune system of the patient.

Example 5: The limits of sensitivity of the multiparameter antibody analysis

A standard series of dilutions of pure human IgG are spotted on to Millipore sheets as in Example 1. The sheets are then processed in exactly the same way as in Example 2, using a 1/100 dilution of the same serum as was used in Example 2.

The reflectance of the spots is quantitated using a Thin Layer Chromatogram Scanner, manufactured by the Company "Camag", Muttenz, Switzerland. The following results are obtained.

Amount of IgG in dot (ng)	Reflectance (arbitrary units)
50	54
23.3	49
10.8	41.8
5	37
2.33	22
1.08	14
0.5	10
0.23	7

and, if desired, treated with a blocking solution of horse serum as in the preceding Examples, dried and stored. The individual squares are cut out and placed in the wells of a Costar tray (Cambridge Mass. U.S.A.). Each well is treated with 150 µl of 3% bovine serum albumin, 1% normal goat serum in TBS for about 15 minutes with shaking at ambient temperature. Alternatively, if the Millipore sheet was treated with the blocking solution the wells can be coated separately. The coated trays and filters can also be stored dry as such.

Mice are immunized with the rat brain synaptosomal membrane preparation, the spleens removed, hybridized with myeloma cells, and distributed in 200 wells in selective medium to permit the growth of hybridomas, by known procedures [G. Köhler, C. Milstein, Nature 256, 495-497 (1975)].

After 10 days, aliquots of the supernatant from the wells are placed in the Costar plate wells containing the squares (75-150 ul per well) or dilutions therefrom into blocking solutions, and the antibody binding reaction permitted to continue for between two hours and overnight, depending on the activity of the antibody. The medium is then removed and the excess antibody removed by extensive washing with TBS. The bound immunoglobulin is then specifically stained with peroxidase-coupled goat-anti-mouse IgG, using the same procedure as in the preceding Examples. Out of the 480 wells 170 positives are detected.

This procedure can be used for the preparation of kits for the screening of hybridomas against any desired antigens or mixtures of antigens. It has the advantage of permitting the easier handling and storage of the antigen if it is immobilized on sheets or squares rather than on conventional plastic dishes, it requires less antigen than if the entire well of the Costar dish is coated with the antigen, and it permits the direct comparison of the antibody binding to the antigen compared with the background staining on the Millipore, thus permitting highly sensitive discrimantion and elimination of false positive due to high background reactions.

The samples are then washed with TBS and incubated with a 1/1000 dilution in TBS-10% horse serum of peroxidase coupled rabbit anti-human immunoglobulins for the human sera and rabbit anti-mouse immunoglobulins for the mouse sera. The last two detection antisera are obtained from DAKO, Copenhagen, Denmark. They are then incubated for a further 2 hours at ambient temperature. The excess detecting antibody is then washed out with TBS and the bound antibody detected with the chloro-naphthol reaction, as in Example 5. The color is allowed to develop for a further 2 hours and the results interpreted.

Both the human autoimmune sera show significant rheumatoid factor down to the 1:1000 dilutions, and none is seen at any dilution in the control serum. The mice autoimmune serum show a positive reaction down to the 1:10000 dilution, and the control mouse serum show a borderline-detectable positive reaction with the 100-fold diluted serum. The assay thus detects the presence of the high titer anti-rabbit IgG antibodies in both the pathological human and mouse sera. Immune complexes are also detected in the pathological sera at titers of approximately 10 times the control sera. High titer circulating immune-complexes are found in the auto-immune mouse sera and the human MCTD and SLE sera. Further examples with a collection of two hundred human SLE sera and 20 individuals MRL mice with sera samples taken throughout their life-time, support these results.

The conditions described in this Example thus provide a workable system for detecting rheumatoid factors and immuno-complexes in both clinical diagnosis and animal model systems.

Example 10: Different microporous materials as supports for the dot immuno-binding assay

A variety of microporous support materials are used, and 0.5 µl samples of antigens are applied as in the preceding Examples:

Supports: (1). New England Nuclear Cor., Boston, Mass. USA "Gene Screen" (microporous polyamide)

TBS is the solvent as elsewhere.

After addition of the DNA samples, and before addition of the remainder, the blots are baked at 80° for two hours.

The following results are obtained and are compared with the standard system using Millipore of 0.45 μ pore size:

Support	Results
(1)	Faintly visible A, C and F. Other dots negative.
(2)	A, B and C stain, D, E, F and G negative.
(3)	All dots positive, D, E and F show a series of graded intensity.
(4)	A and C faintly positive, all others negative. Background higher.
(5)	No significant difference from Millipore.
(6)	Exactly same as Millipore of same porosity.
(7)	D does not stain, otherwise as Millipore.
(8)	A, B and C stain, D, E, F fainter than Millipore.
(9)	A, C and F stain faintly. Others negative.
(10)	A and B positive, C faint, others negative.
(11)	A, B and C positive but faint, D, E, F borderline visible.
(12).	A, B and C positive, D, E and F weakly positive.

Agar has the merit of being easily coated on to a robust support and is transparent, so lending itself to quantitation by transmission instead of the reflectance measurements as in Example 5.

Summary of respiratory virus antibody profiles obtained by dot-immuno assay.

serum code	Titer of antibody in µg/ml in original serum obtained with antigen										
	1	2	3	4	5	6	7	8	9	10	11
1	0.1	<0.03	0	<0.03	0.15	0.15	0.03	0.15	0.1	0.03	0.33
2	0.07	<0.03	0.07	0.03	0.15	0.07	0.07	0.07	0	0	0.33
3	0.03	0	0	<0.03	0.15	0.15	0.07	0.07	0.03	0	0.33
4	0.07	0.15	0.07	<0.03	0.15	0.15	0.15	0.07	0.03	0	0.72
5	0.03	0	0.07	<0.03	0.03	0.07	0.07	0.07	0	0	0.72
6	0.07	0.03	0	<0.03	0.15	0.1	0.07	0.07	0	≪0.03	0.72
7	0.07	2.3	<0.03	<0.03	0.03	0.15	0.07	0.07	0.1	<0.03	0.33
8	0.03	<0.03	0	<0.03	0.03	0.15	0.07	0.07	0.5	3.3	0.33
9	0.03	0.03	0	<0.03	0.03	0.03	0.07	0.07	0.03	<0.03	1.5
10	0.03	≪0.03	0.15	≪0.03	0.03	0.07	0.07	0.07	0.03	0	0.33
11	0.03	≪0.03	0	≪0.03	0.03	0	0.07	0.07	0	0	0.33
12	<0.03	≪0.03	0	≪0.03	0.03	0.15	0.07	0.07	0	0	0.33
13	0.03	≪0.03	0.03	<0.03	0.07	0.07	0.07	0.07	0.72	≪0.03	≪0.33
14	0.03	0.03	0.03	0.03	0.07	0.07	0.07	0.07	0.1	0.03	0.72
15	0.03	<0.03	0.15	≪0.03	0.15	0.15	0.07	0.07	0.1	0.03	0.03
16	0	≪0.03	0	<0.03	0.15	0.5	0.07	3.3	0	0.33	3.3
17	0	≪0.03	0	<0.03	0.15	0.5	0.07	3.3	0	0.33	3.3
18	0.03	≪0.03	0.07	<0.03	<0.03	0.33	0.07	1.5	0.15	0.07	0.7
19	0.07	≪0.03	0.03	<0.03	0.07	0.1	0.07	0.07	0.07	0.03	0.33
20	<0.03	0.5	0.03	<0.03	0.07	0.1	0.07	0.07	0.03	0.03	0.33
21	<0.03	<0.03	0.03	<0.03	0.03	0.03	0.07	0.07	0.03	0.33	0.33
22	0.03	0.5	0	<0.03	0.03	0.03	0.03	0.07	0.03	0.33	0.33
23	0.03	<0.03	0.03	<0.03	0.07	0.07	0.03	0.07	0.15	0.33	0.33
24	0.03	0.03	0.33	<0.03	<0.03	0.07	0.03	0.07	0.15	0.03	0.7
25	0.03	0.15	0	<0.03	0.03	0.07	0.03	0.07	0.03	0.15	0.33
26	≪0.03	0.07	0	<0.03	0.07	0.03	0.07	0.07	0.07	≪0.03	0.15

Correlation of respiratory virus antibody profiles from dot immuno assays with corresponding data from complement fixation assays.

In each case, the left hand column is the order of titers for sera by the dot assay and the right hand column the order by the complement fixation assays. The sera underlined are those which were provided as part of the kit as control positive for that particular antigen.

Adenovirus		Ornithosis		Influenza A		Influenza B		Para influenza 1	
1	19	7	7	2	14	1	16	16	16
2	6	20	25	14	2	2	17	17	17
4	7	22	20	1	5	3	18	18	18
6	9	4	1	3	9	4	15	1	25
7	8	25	2	4	4	6	19	3	7
19	1	26	3	5	7	15	25	4	8
3	2	6	4	6	21	16	4	7	11
5	5	24	5	7	25	17	3	8	12
8	25	1	6	8	1	18	7	12	1
9	21	2	8	9	6	13	20	15	2
10	3	8	9	13	8	19	6	6	3
13	4	14	10	16	10	20	1	19	4
14	15	15	11	17	3	23	9	20	5
15	18	21	12	18	11	26	21	2	6
18	10	23	13	19	12	14	8	5	9
22	11	9	14	20	13	5	2	10	10
23	12	10	15	21	15	7	5	13	13
24	13	11	16	22	16	8	10	14	14
11	14	12	17	23	17	9	11	23	15
12	16	13	18	24	18	10	12	24	19
20	17	16	19	25	19	11	13	25	20
21	20	17	21	26	20	12	14	26	21
25	22	18	22	10	22	21	22	9	22
26	23	19	23	11	23	22	23	21	23
16	24	3	24	12	24	24	24	22	24
17	26	5	26	15	26	25	26	11	26

It can be seen from the tables that in almost all cases, the positive sera fall into approximately the same rank, and there are even cases where both assays demonstrate that the serum provided as positive is in fact negative or of low titer. All assays correlate rather well. The reasons for the exceptions may be that the 2 assay systems recognize different classes of antibodies and that the antigen is presented to the antibody in a very different form in the two assays.

Example 13: Use of a dot assay for the typing of monoclonal antibodies.

Monoclonal antibodies to ribosomal proteins from chick liver are prepared by the same procedure as in Example 8, and the specificity of the antibodies determined by the method published by Towbin et al (Proc. Nat. Acad. Sci. U.S., 76, 4350-4354 (1979)). The supernatants from the hybridoma cultures are tested for the antibody type by the following dot assay. Type-specific goat anti-mouse immunoglobulin antibodies from Nordic are dissolved according to the manufacturer's instructions, diluted 30-fold and 1 µl aliquots dotted on to nitrocellulose, the strips blocked with horse serum as in Example 1, incubated overnight at room temperature with undiluted hybridoma supernatants, then bound antibody detected with immun-peroxidase staining as in Example 1. The results are shown in the following Table:

	Stain with antibody against	
Monoclonal antibody	IgG	IgM
Anti-S6	+	-
Anti-L7	-	+
Anti-L18a	+	-
Anti-P1/P2	-	+
Anti-rRNA	-	+

After indefinite storage, the above kits may be used as follows for the analysis of unknown antibodies in serum, corresponding to the methodology of the preceding Examples. One portion each of A, B and C is reconstituted with 100 ml of distilled water. A is then used in the dilution of the unknown sera, B for the indicator antibody binding reaction, and one lot of reconstituted C, together with one ampoule each of D and E make up the color reaction mixture. Other lots of C are reconstituted as needed for the washes after each stage of antibody binding.

The same results are obtained as in the preceding Examples.

6. A device as claimed in claim 4, wherein the sheet has a thickness of approximately 0.1 mm.

7. A device as claimed in claim 1, wherein the material of the solid support is a member selected from the group consisting of
A) Natural polymeric carbohydrates and their synthetically modified, crosslinked or substituted derivatives, selected from the group consisting of a) agar, agarose; cross-linked alginic acid; substituted and cross-linked guar gums, cross-linked dextran polymers and starches b) regenereated celluloses; cellulose esters, mixed cellulose esters, cellulose ethers.

B) Natural polymers containing nitrogen, selected from the group consisting of proteins and their derivatives,

C) Natural hydrocarbon polymers selected from the groups consisting of latexes and rubbers.

D) Synthetic polymers which can be prepared with suitably porous structures selected from the group consisting of a) vinyl polymers and their partially hydrolysed derivatives, polyacrylates, polyacrylamides, polymethacrylates b) copolymers and terpolymers of the above vinyl monomers among themselves and with other monomers c) polyesters and polyamides d) polyurethanes or polyepoxides.

E) Inorganic materials which can be prepared in a suitably porous form, selected from the group consisting of sulfates or carbonates of alkaline earth metals and magnesium, silicates of alkali and alkaline earth metals and/or aluminium and/or magnesium, aluminium or silicon oxides or hydrates,

F) Mixtures or co-polymers or graft co-polymers of the above classes.

8. A device as claimed in claim 7, wherein the material of the solid support is a cellulose ester with nitric acid or with an aliphatic carboxylic acid having from 1 to 7 carbon atoms, or a mixture of such esters.

18. A device as claimed in claim 2, wherein nucleic acids are applied to the solid support, followed by baking the support at temperatures in the range of 60° - 120°C, for a duration ranging from 5 minutes to 12 hours.

19. A kit as claimed in claim 1, comprising a device in the form of a solid support prepared with antigens or immunoglobulins and optionally blocking proteins, or combinations thereof, suitable hardware for the performance of the immunological reactions, and reagents for an indicator system in pre-aliquoted or dessicated form.

20. A kit a claimed in claim 19, comprising an indicator antibody radioactively labelled where detection and quantitation is to be carried out by counting or autoradiography; or conjugated with a fluorescent indicator where detection and quantitation is to be carried out by fluorimetry; or conjugated with an enzyme capable of giving a color reaction with a suitable substrate where detection and quantitation is to be carried out by densitometry or visually; or comprising a detection and quantitation system based on complement protein binding to antigen-antibody complexes, where the complement itself is labelled by any one of the above three methods or by means of a further specific anti-complement antibody, also labelled by any one of the above three methods.

21. A kit as claimed in claim 19, wherein the hardware comprises a multi-cavity plastic tray, and the reagents are provided in the form of a lyophilized mixture of indicator antibody, salts, buffers, carrier serum or protein, and pre-determined amounts of indicator enzyme chromogenic substrate, salts, buffers, and ampoules containing pre-measured volumes of liquid substrates, all in suitable packaging.

by immunoassay methods for the diagnosis, surveillance and prognosis of diseases in humans and animals.

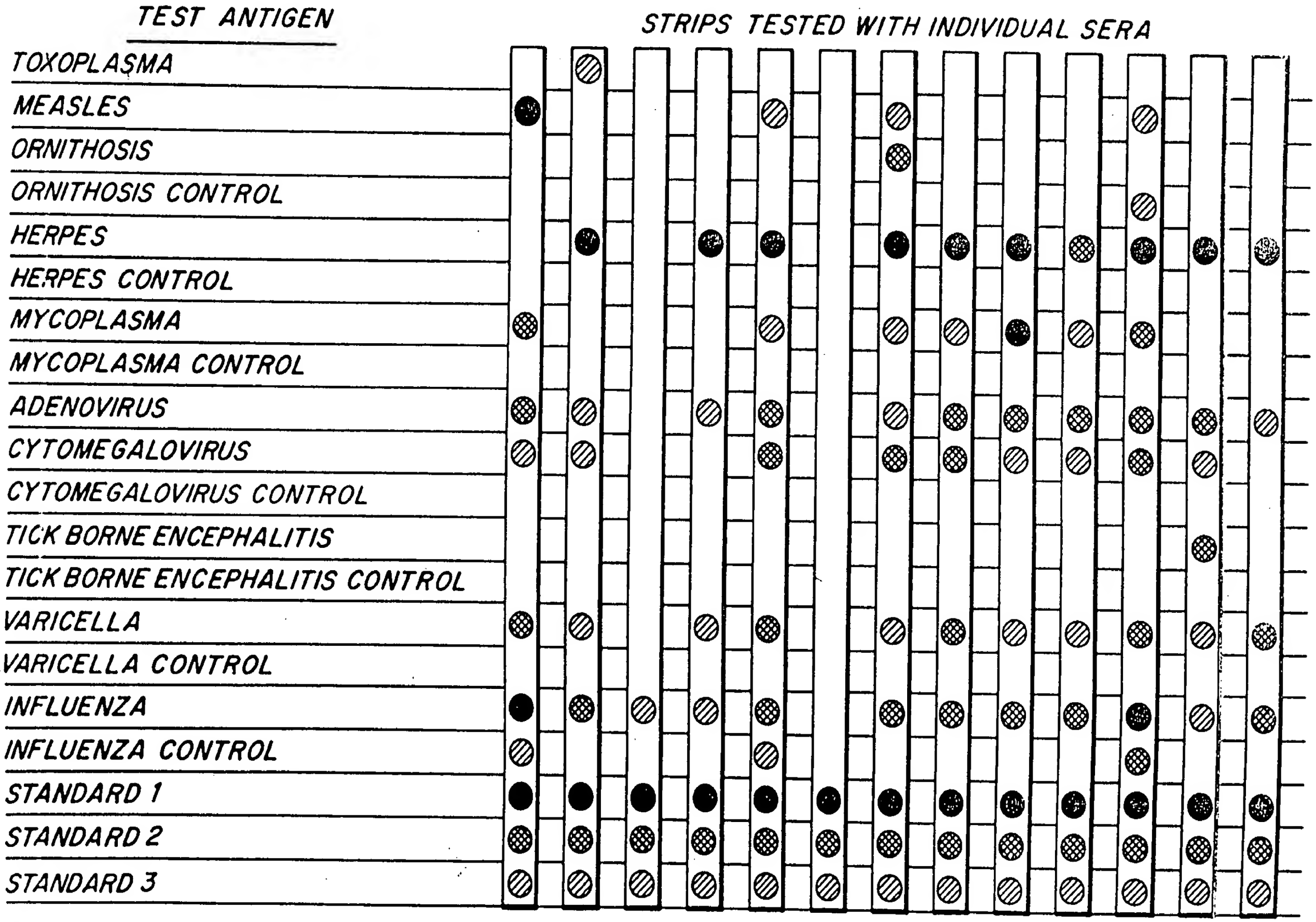
30. The use of devices or kits as claimed in claim 29 for the screening, detection and quantitation of monoclonal and other antibodies or antigens in research and development.

31. The use of devices or kits as claimed in claim 29, wherein unknown antigens are applied to the solid support and are detected and quantitated with immuno-assay methods using known antibodies.

32. A process for the manufacture of a device as claimed in anyone of claims 1-18, wherein aliquots of solutions or suspensions of one or more antigens or immunoglobulins or of both of them are applied by direct contact to a solid porous support to form a pre-selected array, and, if desired, the device so obtained is treated with proteins which are non-specific with regard to their capacity of reacting with the mentioned antigens or immunoglobulins to saturate residual adsorption sites inside or outside ~~ourside~~ the antigen or immunoglobulin areas.

33. Process for the immunological analysis consisting in incubating any of the devices or kits claimed in anyone of claims 1 - 28, and having been suitably treated with a blocking solution containing non-specific proteins to saturate all residual binding sites, with the sample containing the immunological factor to be detected, preferably in the presence of blocking solution, and, if desired, after washing, with a solution of the indicator antibody or any signalling system for recognizing the immunological complex formed by the primary incubation with the sample, also preferably in the presence of blocking solution, and development of the indicator system and/or quantitation of the immunological factor is carried out.

TEST STRIPS FOR ANTIBODY-ANALYSIS



The standards give three gradations of color intensity : ●, ◍, ◍.
Antigen spots are matched with the corresponding standard intensity.

European Patent Office

EUROPEAN SEARCH REPORT

0063810
Application number

EP 82 10 3520

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
Y	FR-A-2 257 259 (GENERAL ELECTRIC COMPANY) * Page 3, lines 23-38; page 4, lines 1-38; page 5, lines 1-38; page 6, lines 1-38; pages 7-10; figures 2,6 * & GB - A - 1 486 826	1,3,4, 14-16, 29,31-33	G 01 N 33/54 G 01 N 33/56
D,Y	US-A-4 200 690 (ROOT et al.) * Abstract; column 5, lines 40-68; column 6, lines 1-3, 63-68; column 7, lines 1-9; claim 1 *	1,4	
Y	US-A-4 157 895 (FINLAY et al.) * Abstract; column 1, lines 19-39; column 2, lines 29-62; column 4, lines 21-68; column 5, lines 1-27; claim 4 *	1,7,8, 11,14, 22	TECHNICAL FIELDS SEARCHED (Int. Cl. ³) G 01 N
Y	DE-A-2 638 207 (GENERAL ELECTRIC) * Page 6, line 31 - page 9, line 14; claims 1-54 * & GB - A - 1 564 333	1,3,4, 14-16, 29,31-33	

-/-

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	30-07-1982	DE LUCA G.M.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
D,A	EP-A-0 027 008 (VENTREX LAB.) * Abstract; page 14, lines 17-38; page 15, lines 1-19 *	7-9,20	
A	US-A-3 664 421 (PRICE) * Abstract; column 2, lines 35-60; column 3, lines 1-14, 49-72; column 4, lines 63-75; column 5, lines 1-5; claims 1,2,4 *	11,14	

TECHNICAL FIELDS SEARCHED (Int. Cl. ³)

The present search report has been drawn up for all claims

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T : theory or principle underlying the invention
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& : member of the same patent family, corresponding document

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